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Biochar amendment of a sandy loam improves wheat growth under drought and control conditions

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Abstract

Drought stress is a major limiting factor in cereal crop growth. To meet the feeding requirements of an increasing population, cereal crop yields need to be significantly increased in a sustainable manner. Biochar is a charcoal-like substance derived from organic material. Here, the effects of biochar on wheat (*Triticum aestivum* L.) growth and stress responses under drought stress conditions were determined. Biochar was applied at 5% (*w/w*) and stressed plants weren't watered during the four weeks of drought treatment to establish the rate of water loss in the different soil treatments. drought significantly decreased plant height, stem thickness, number of tillers, and shoot dry biomass, relative water content, and quantum efficiency of photosystem II in wheat. Biochar application significantly increased plant height, stem thickness, root and shoot dry biomass, and the quantum efficiency of wheat under drought stress and in the control. An interaction between drought and biochar seen in the relative water content along with the increased water use due to biochar suggests an improved water availability when biochar is applied. I determine that biochar is a sustainable soil amendment for improving plant growth under drought conditions, but only to a point, potentially making it unsuitable for use in areas that experience long term drought and aren't irrigated. In irrigated areas, biochar has the potential to reduce water use by allowing for a reduced watering frequency, improving water use efficiency while mitigating any yield reductions due to an induced water-deficit due to reduced watering frequency.

Keywords: Biochar, wheat, *Triticum aestivum* L., drought, sandy loam, food security, soil degradation, soil carbon.

Introduction

Food shortages occur regularly around the world with over one billion people unable to access the required calories leaving them undernourished (Mc Carthy *et al.*, 2018). Current predictions estimate world population in 2050 to be in excess of nine and a half billion, further stretching our limited food production capacity (United Nations, 2019). By 2050 we must increase food production by 70% globally and up to 100% in developing nations to meet the needs of the increased population (FAO, 2011; Mc Carthy *et al*., 2018; van Dijk *et al*., 2021). Furthermore, food shortages, especially in developing nations, can lead to social unrest and in some cases be a precursor for war (Bello and Baviera, 2009; Frankelius, 2019; Soffiantini, 2020) further highlighting the importance of maintaining food security.

Between 1960 and 1999, a population boom from 3 billion to 6 billion people occurred, with slower growth since (United Nations, 2019), facilitated by increased crop yields because of rapid technological developments in agriculture. This period of increased farming intensity was characterised by the development of high yielding crop varieties through selective breeding, increased use of agrochemicals such as pesticides and synthetic fertilisers, and the increased mechanisation of farming (Evenson and Gollin, 2003; Lam, 2011). However, such developments have left our soils structurally and biologically degraded primarily due to the degradation of soil organic matter (SOM) into carbon dioxide as a result of the overploughing of fields (Oldeman, 1992; Stockfisch *et al*., 1999). The increased release of greenhouse gases from agrochemical production, machinery use, ruminants, and the breakdown of SOM, among other processes, meant that in 2018, agriculture alone (not including fisheries or other land use) emitted 17% of global greenhouse gas (Johnson & Johnson, 1995; FAO, 2020) making it one of the top contributors towards climate change.

Neither the increased use of agrochemicals such as pesticides and synthetic fertilisers or increased soil disturbance due to increased mechanisation of farming are sustainable solutions for improved crop yield (Evenson and Gollin, 2003; Lam, 2011). Furthermore, simply expanding total farmed acreage won't be enough to meet our needs: in 2019, agricultural land use accounted for approximately 37% (47.5 million km^2) of total terrestrial land and around 50% of habitable land (FAOSTAT, 2019). Considering the required increase in crop production and limited availability of arable land, cropland expansion is unsustainable. Instead, increases in crop yield from existing agricultural land are required (Foley *et al.,* 2011). Currently, yearly yield increases of the primary cereal crops: maize, wheat, soy and rice, which contribute roughly 66% of global calory intake, are at 1.6%. This is below the required 2.4% needed to meet future demands to roughly double production by 2050 (Ray *et al*., 2013; FAO, 2017). More specifically, increased production of wheat is fundamental to food security. It is the most abundant cereal crop by farmed acreage on the planet (Shiferaw *et al*., 2013) and provides roughly 20% of global daily calories for humans as well as being a dense source of dietary components such as B-vitamins, fibre, proteins, and minerals (Ahmad *et al.,* 2018).

Two major barriers to increasing wheat crop yield are water deficits and soil degradation (Siddique *et al.,* 2000; Bot and Benites, 2005), both of which interact with each other. Increased intensity and frequency of extreme weather events, such as drought, are a direct result of climate change (Trenberth *et al*., 2014; Mukherjee *et al*., 2018). The severity of drought conditions is compounded by poor soil conditions due to soil degradation, meaning when it does rain, less water is available to crops due to poor water retention and low levels of infiltration (Bot and Benites, 2005).

Drought stress in wheat manifests as numerous physiological effects (Anjum *et al.,* 2011; Ahmad *et al.,* 2018). Plants experiencing drought stress see a reduction in electron transport and so a reduction in the rate of photosynthesis (Ahmad *et al.,* 2018). Disturbance in electron transport also leads to the increased production of reactive oxygen species which cause oxidative stress to photosystems, reducing photosynthetic efficiency, and lipid peroxidation of cell membranes reducing membrane stability (Wang *et al.,* 2014). Reduced plant water levels lead to a build-up of solutes in the leaves which reduces leaf water potential (Nawaz *et al.,* 2014). Lower leaf water potentials lead to reduced stomatal conductance and rate of transpiration due to the closing of stomata as a stress mitigation response. This reduces the rate of photosynthesis (Siddique *et al.,* 2000; Liang *et al.,* 2002) and so the fixing of carbon, reducing growth. The rate of photosynthesis is also reduced due to a reduction of chlorophyll content because of drought stress (Fotovat *et al.,* 2007). Reduced water availability because of drought conditions causes plants to prioritise deeper root growth in search of water through abscisic acid signalling from the roots, reducing shoot growth and yield potential (Hsiao and Xu, 2000; Ahmad *et al.,* 2018). Overall, drought stress in wheat leads to an overall reduction in growth and yield due to the use of energy in stress mitigation as well as a lack of water for fundamental physiological processes (Ahmad *et al.,* 2018).

Globally, between 1983 and 2009 75% of harvested wheat cropland experienced drought induced yield loss with each drought event leading to an average yield loss of 8%, higher than rice (3%) and maize (7%) (Kim *et al*., 2019). Such differences are likely due to the increased sensitivity of wheat cropland to drought due to the reduced proportion of irrigated land compared to other crops (Geravandi *et al.,* 2011; Kim *et al*., 2019). It is possible yield losses are even greater in *Triticum aestivum* L. compared to other wheat varieties due to its cultivation in many semi-arid regions (Geravandi *et al.,* 2011). Yield loss directly due to soil erosion is difficult to quantify, however, it is considerable (Bindraban *et al*., 2012). Increasing irrigation of wheat crop land is not sustainable and intensification of irrigation post WWII has already had destructive effects on ecosystems worldwide and continued over-exploitation and eventual depletion of these resources would have a devastating impact on future crop yields (Stockle, 2001). For instance, groundwater pumped from aquifers supplies 60% of irrigation in the United States. Current predictions suggest around a third of the southern High Plains, an important area for US wheat production, will no longer be able to support current irrigation pumping requirements within the next 30 years, hampering required yield boosts (Scanlon *et al*., 2012). However, concerns over ground and surface water overexploitation are not confined to the US (Madramootoo, 2012; Dassi *et al*., 2018). In Mexicali, Mexico, the little remaining water of the Colorado river not used for western US agriculture is diverted from its natural route to irrigate local farms. Predictions of increased upstream water use for agriculture puts the livelihoods of those who rely on the waters for irrigation in Mexicali at stake (Summitt, 2013).

Soil biota are essential for the breakdown and incorporation of organic matter into the soil, nutrient cycling, soil aeration and reduction of bulk density through burrowing by annelids and biotic-biotic interactions such as those between mycorrhizal fungi and most plant species (Dick, 1992; Bonfante and Anca, 2009). Improving soil structure, by way of soil amendments and biotic processes, has the potential to reduce the need for irrigation by reducing runoff after rain, locking in more water to the soil and increasing availability of soil moisture to plants (Bot and Benites, 2005). Numerous factors affect soil physical and biological properties. Soil texture has a major impact on soil water properties with clay rich soils holding more water for longer compared to sandier soils due to the reduced particle and pore size in clay soils restricting water flow (English *et al*., 2005). SOM content affects soil water properties through increasing aeration and porosity of soils improving water infiltration (Bot and Benites, 2005). It also increases soil water retention. However, SOM decomposes easily by way of biotic and abiotic processes. If the rate of addition drops below the rate of decomposition, as is the case in many agricultural soils (Stockfisch, 1999), soils become depleted.

Biochar is a renewable, low-density charcoal made from heating organic material in a low-oxygen environment in a process called pyrolysis (Tagliaferro, 2020). Over the last two decades there has been a focus on biochar as a soil additive for improving soil properties (Lehmann *et al*., 2006; Wang *et al*., 2016) by reducing chemical inputs and increasing carbon stocks. There is a vast array of feedstocks used to produce biochar including animal manure, crop stubble, wood chips, and papermill sludge (Singh *et al.,* 2010). However, all materials used are renewable and often waste products (Ippolito *et al.,* 2020). The process of pyrolysis itself requires very little external energy input as the reaction becomes self-sustaining once the correct temperature is reached making it an environmentally and economically sustainable soil additive (Miles, 2020).

Biochar has been shown to have numerous effects on soil chemical, biological and physical properties that lead to increased crop yields (Miles, 2020). Its addition increases the cation exchange capacity (CEC) of soil increasing nutrient retention reducing leaching while simultaneously increasing nutrient availability to plants (Agegnehu *et al.,* 2015) and fertilising the soil due to the high ash content which itself increases the pH of the soil, reducing the availability of toxic metals and acting as a pH buffer, stabilising soil pH (Wacal *et al.,* 2019). The porosity of biochar improves oxygenation of soil (Manariotis *et al.,* 2015) and provides a habitat for microbial life promoting its activity (Kumputa *et al.,* 2019). Biochar has also been shown to increase the stability of SOM, further compounding SOM's beneficial effects while the biochar itself exhibits a high stability allowing it to last for centuries in soil (Wang et al., 2016). Biochar has also been shown to affect plant- and soil-water interactions. The pore spaces within biochar can hold more water compared to simple adhesion of water to soil particles in both medium- and course-textured soils due to capillary action (Razzaghi *et al.,* 2020). This increases the field capacity of soil increasing reserves of water during periods of drought and holding more in the upper horizons of soil where the bulk of root systems exist (Fan *et al., 2016*). Furthermore, biochar has been shown to improve availability of water to plants (Ma *et al.,* 2016).

Improved water availability coupled with increased soil field capacity should result in improved resilience to drought stress in plants manifesting improved crop growth and yield. This has been supported by numerous studies with wheat (Abbas *et al.,* 2018; Haider *et al.,* 2020; Khan *et al.,* 2021; Zaheer *et al.,* 2021) as well as various other crops (Kammann *et al.,* 2011; Ahmed *et al.,* 2016; Hashem *et al.,* 2019). However, little focus has been put on the effect of biochar on drought stress specifically on the early growth stages of wheat. Latinini *et al.* (2021) reported reduced growth in early-stage Durum wheat under regular water conditions when biochar was applied contrasting with the improved growth seen in developed wheat.

Strong early-stage development is important in wheat for resistance against physical stressors such as wind and hail as well as reducing the likelihood of plant death from pests (Changnon, 1972; Cleugh *et al.,* 1998). This study focuses on the effects of biochar application to an agricultural soil on the early-stage development of Tybalt spring wheat under stress conditions through measuring several plant stress and growth attributes.

Experimental procedures

Experimental design

Biochar was purchased from the UK based company SoilFixer. Mixed European hardwoods were used as the feedstock for biochar production. The material was pyrolysed between 500-600°C for 3.5 hours in a retort-kiln with the resulting product sifted to size ranging from 2-8mm. Tybalt spring wheat (*Triticum aestivum* L.) seeds were purchased from the seed breeding company Limagrain UK Ltd. They were grown in soil originating from a Devon farm field in the southwest UK used annually for crops. Growth environment was a greenhouse in Skardon Gardens, Plymouth UK with supplemental lighting to provide 16 hours of light per day. During the growth period (October to December 2021) the temperature ranged from 7.8°C to 27.5°C with a mean of 12.9°C while humidity ranged from 34.6% to 100.0% with a mean of 81.7%. The seeds were germinated in a dark heated unit at 25°C for 48 hours on damp tissue in open petri dishes. Seeds with radicles measuring 10mm ±2mm in length were selected for uniformity and planted directly into 10.7 L troughs measuring 12 cm (W) by 53 cm (L) by 17 cm (H) 7 cm apart in two rows with a total of 12 plants per trough. Four troughs were filled with unamended soil while four were filled with a 5% (*w/w)* biochar-soil mix that was homogenised for three minutes. This is equivalent to approximately 70 Mg ha^{-1} when applied to the top 17 cm of soil (the height of the troughs used). All soils were enriched with slow-release fertiliser pellets (NPK: 12-7-9) as per the instructions from the producer, Miracle Grow.

Trough positions were changed several times throughout the experiment to reduce variation from differing exposure to heat and solar radiation within the greenhouse. After 31 days since treatment initiation, the wheat was harvested. Troughs were then lightly shaken to break up the soil. A trowel was used to ease the plants, with the roots, out of the soil. The roots were separated from the stem and rinsed ready for further processing. From planting of the germinated seeds into the troughs, the wheat was left to grow at 100% field capacity for one week. After this, two troughs of each soil type were subjected to drought stress treatment while the remaining four (two of each soil type) were controls for a total of two replicates for each unique treatment. Controls were kept between 80% and 100% field capacity by weighing twice a week and watering as needed to achieve 100% field capacity. Field capacity was calculated as described in Michael *et al.* (2017). Contrary to other drought stress works (Farzad et al., 2011; Samarah, 2005; Snow and Tingey, 1985) where the researchers maintained the drought stressed soils at a set field capacity, our drought stressed soils were watered to 100% field capacity on the first day of the stress regime and weren't watered again throughout the growing period. This was done to analyse any differences in the drainage rates between soil with and without biochar. Trough weights for all troughs were recorded twice a week to keep track of the rate of water loss from the soil.

Stress Measurements

Quantum efficiency of photosystem II (Fv/Fm)

Fv/Fm measurements were taken using the Pocket PEA Rapid screening continuous excitation chlorophyll fluorimeter from Hansatech Instruments. Four plants from each trough were selected for sampling using a random number generator to select each plant. The same plants were then used for each measurement over time. The lowest leaves on each plant were used as they were the largest so would fill the PEA meter dark adaption clips. Two measurements were taken a week apart at midday, with the last being the day prior to harvest. Earlier measurements could not be done due to insufficient leaf size for the clips. Conditions during both measurements consisted of light cloud cover.

Relative water content (RWC)

Immediately prior to harvest, a 10 cm length of leaf, measured from the tip down, was taken from the upper and lowermost intact leaves from two plants per trough selected by random number generator. RWC was calculated using the standing rehydration technique as outlined in the literature (Lafitte, 2002; Sanders and Arndt, 2012). Leaf samples were put cut end down into 50 mL Falcon tubes containing 1mL distilled water and left in a dark fridge overnight. The standing rehydration technique was chosen due to its tendency to produce the fewest errors compared to other rehydration techniques (Arndt *et al.,* 2015).

Proline Quantification

Proline quantification was done according to steps detailed in the literature (Carillo and Gibon, 2011; Nisha *et al.,* 2016). Immediately prior to harvest ~200mg of leaf tissue was taken from two plants from the lowest leaves (selected using a random number generator) from each trough and weighed. Each sample was combined with a 40% ethanol and 10mM ascorbate mixture using a pestle and mortar and then refrigerated for 20 hours in Eppendorf tubes. The samples were then centrifuged at 13kRPM for 10 minutes. Extract and glacial acetic acid were pipetted into one set of Eppendorf tubes and extract and 1.25% w/v ninhydrin reagent in glacial acetic acid into another. These, along with the proline standards and blank were placed into a heat block at 100ºC for 30 minutes before being run through a plate spectrophotometer at 520nm.

Growth Measurements

Plant height from soil level to the tip of the longest leaf was measured every seven days. One day prior to harvesting, stem thickness was measured using a set of callipers one centimetre from soil level. Five plants from each trough were randomly selected using a random number generator to select from their assigned numbered positions. The same plants were then used for each measurement over time. The number of primary and secondary tillers were also counted on all plants. To measure total biomass and derived measurements from biomass, such as root to shoot ratio, all plant material from harvest was dried in an oven at 80°C until no weight change after 12 hours was detected.

Soil analysis

Soil samples were dried in an oven at 80°C for 24 hours and then weighed every 12 hours after until no further weight change was recorded. These samples were sealed in airtight bags to prevent ingress of moisture. Control soil samples were saved for use in texture analysis. Soil texture was determined by analysis done using the Malvern Mastersizer 2000 coupled with the Hydro-G wet sample unit using the following settings: pump at 2500rpm, stirrer at 950 rpm and ultrasonic dispersion for 90s at 90% prior to measurement running software version: 5.6. For calculations, the general analysis model with enhanced sensitivity and irregular particle shape were used with an assumed refractive index of 1.53 and light absorption of 0.01 to 0.001. Three repeat runs in the Mastersizer were done with a total of 5 replicates within each run for a total of 15 results.

Statistical Analysis

Results were presented as mean values. Two-way analysis of variance (Two-Way-ANOVA) was used for all data. Tukey's honest significance difference (Tukey's HSD test) was applied to all two-way-ANOVA results to determine statistical significance of individual treatment combinations. All tests were carried out using R-Studio (R Core Team, 2021).

Results

Plant growth

Growth in terms of both stem thickness and plant height saw a significant overall increase from 2.69 mm ±0.08 to 3.11 mm ±0.14 and 442 mm ±6 to 463 mm ±7 respectively with the addition of biochar to the soil in both drought and control conditions while, as expected, the drought treatment led to an overall reduction in stem thickness from 3.18mm ±0.11 to 2.62mm ±0.09 and plant height from 475mm ±5 to 429mm ±6 in soils both with and without biochar (Figures 1A and 1C). On the contrary, application of biochar to soil had no significant effect on tiller count in either control or drought conditions while exposure to drought stress saw a significant reduction in average treatment tiller count from 24 ±3 to 7.5 ±0.5 in both soils (Figure 1B).

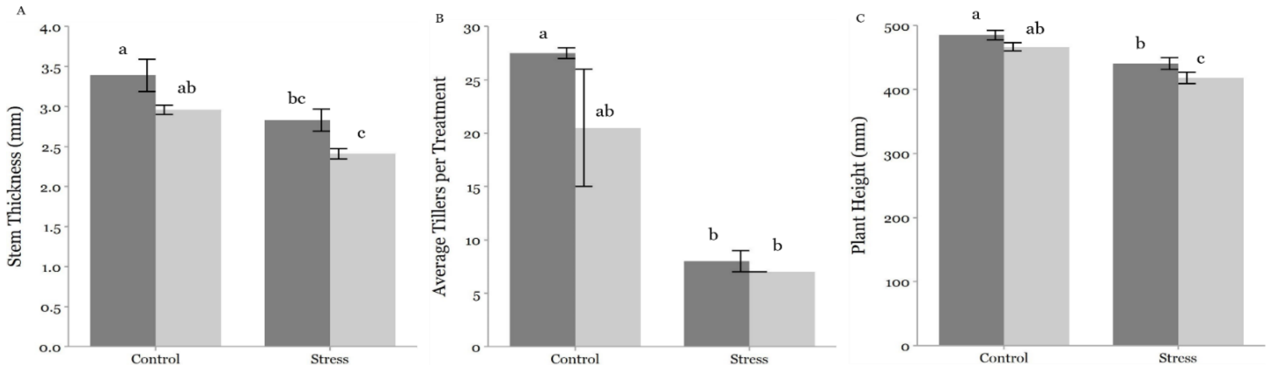

Figure 1: Stem thickness (A), number of tillers (B), and plant height (C) of Tybalt spring wheat grown in a sandy loam treated either with biochar (dark grey bars) or without (light grey bars) after four weeks of growth under well-watered (control) or drought (stress) conditions. Values are means ± SEM (n = 5 for A), (n = 2 for B), and (n = 24 for C). Statistically different ($P \leq 0.05$) means between different biochar and drought treatments calculated using Tukey's-HSD test are indicated by different lowercase letters on the bars.

Biochar led to a significant increase in root dry biomass from 0.24 g ±0.03 to 0.37 g ±0.06 while exposure to drought stress had no significant effect on it (Figure 2A). Biochar application significantly increased both shoot dry biomass from 2.02g ±0.27g to 2.67g ±0.45g and total dry biomass from 2.27g ±0.30g to 3.03g ±0.50g while drought stress reduced shoot dry biomass from 2.92g ±0.34g to 1.78g ±0.10g and total dry biomass from 3.29g ±0.39g to 2.01g ±0.12g (Figure 2C and 2D). Root to shoot ratio was not significantly affected by application of biochar and, interestingly, root to shoot ratio was not significantly affected by drought either (Figure 2B).

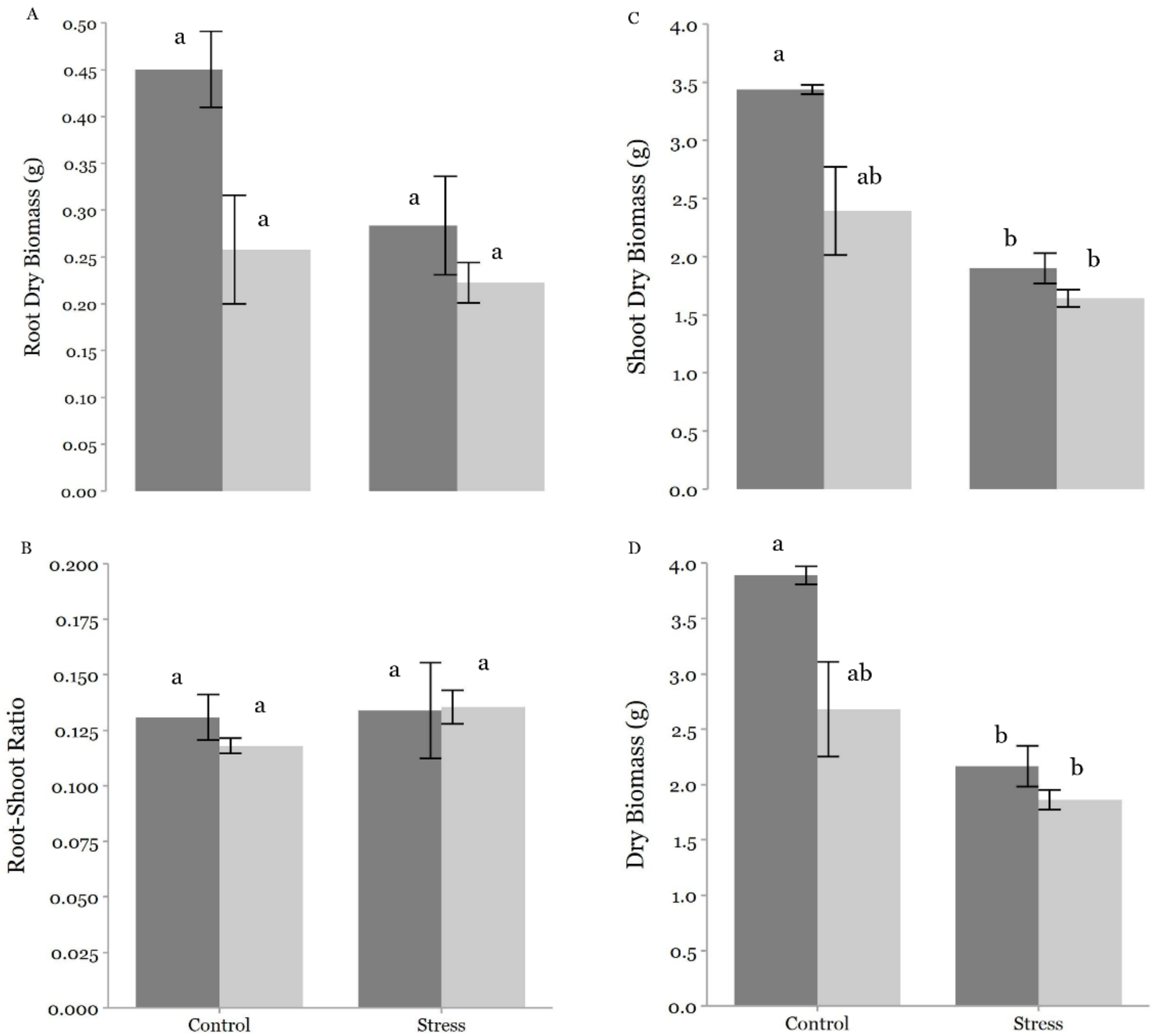

Figure 2: Root dry biomass (A), root to shoot ratio (B), shoot dry biomass (C), and total dry biomass (D) of Tybalt spring wheat grown in a sandy loam treated either with biochar (dark grey bars) or without (light grey bars) after four weeks of growth under well-watered (control) or drought (stress) conditions. Values are means ± SEM (n = 2). Statistically different ($P \leq 0.05$) means between different biochar and drought treatments calculated using Tukey's-HSD test are indicated by different lowercase letters on the bars.

Plant stress physiology

The addition of biochar to soil significantly increased the photosynthetic efficiency of the wheat in both drought stress and control conditions from 0.63 ±0.02 to 0.70 ±0.02 while exposure to drought stress led to a significant decrease in photosynthetic efficiency in both soils from 0.71 ±0.02 to 0.63 ±0.02 (Figure 3A). Relative water content was also significantly reduced in plants exposed to drought stress in both soils from 95% ±0.7 to 90% ±1.1. However, for biochar amended soil, no difference in relative water content was seen in the control. There was, however, a significant decrease in relative water content in the plants grown with biochar compared to those grown without biochar under drought stress from 93% ±0.4 to 88% ± 0.8 (Figure 3B). This lack of significant difference in the control but not in the stressed plants shows a statistically significant interaction between stress and soil.

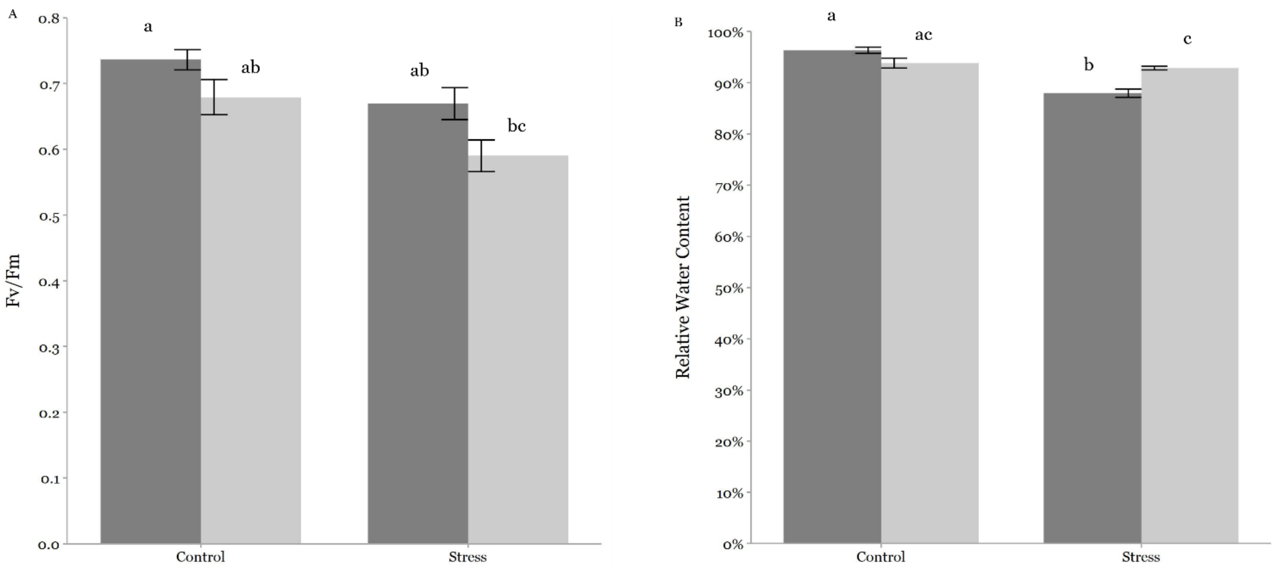

Figure 3: Quantum efficiency of PSII (Fv/Fm) (A) and relative water content (RWC) (B) of Tybalt spring wheat grown in a sandy loam treated either with biochar (dark grey bars) or without (light grey bars) after four weeks of growth under well-watered (control) or drought (stress) conditions. Values are means ± SEM (n = 4 for RWC) (n = 8 for Fv/Fm). Statistically different ($P \leq 0.05$) means between different biochar and drought treatments calculated using Tukey's-HSD test are indicated by different lowercase letters on the bars.

Soil moisture loss

Biochar amended soil under drought conditions had a significantly higher average daily moisture loss (Figure 4) as determined by weighing of the troughs. Because no water was added to the stressed wheat after drought initiation, this led to the biochar amended soil having a lower moisture content by the end of the treatment.

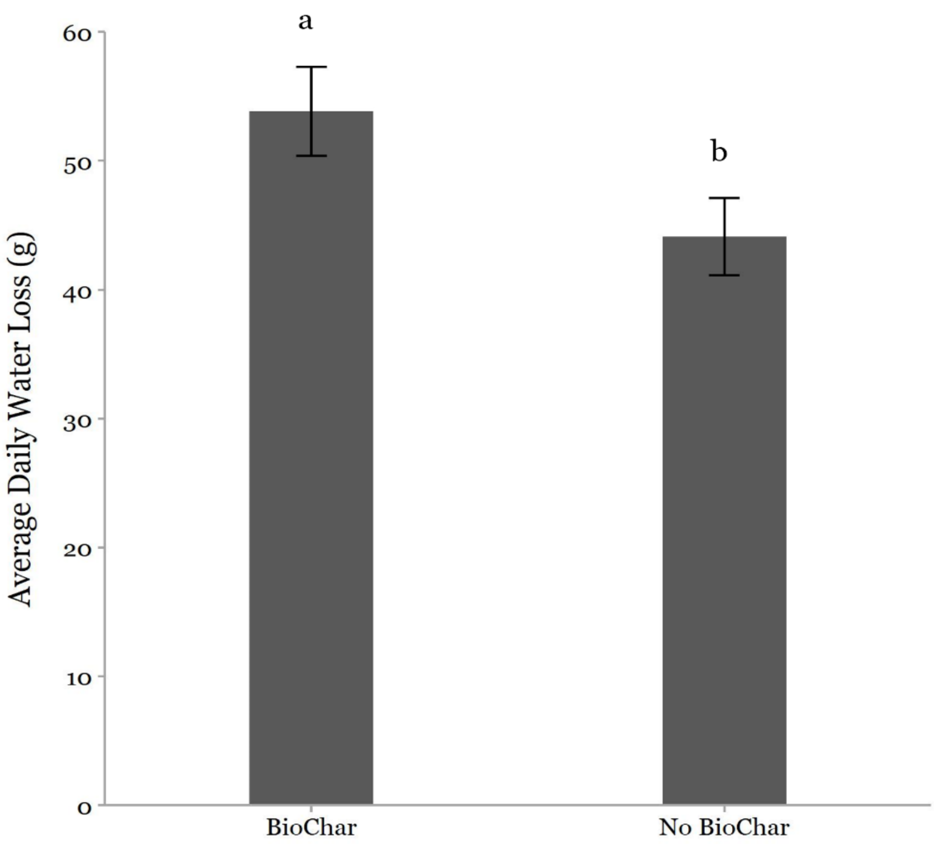

Figure 4: Average daily soil moisture loss from biochar amended and unamended sandy loam soil measured over 28 days. Values are means ± SEM (n = 14). Statistically different ($p \leq 0.05$) means between biochar amended and unamended soil calculated using Tukey's-HSD test are indicated by different lowercase letters on the bars.

Discussion

Plants under drought stress conditions saw a significant reduction in height, shoot dry biomass, and stem thickness. Such findings are supported by the literature in wheat (Abbas *et al.,* 2018; Haider *et al.,* 2020) and other plants (de Silva *et al.,* 2012; Rizwan *et al.,* 2018), while Grzesiak *et al.* (2019) reported an increased or similar number of tillers in drought-resistant wheat varieties and a reduction in drought-sensitive varieties. Loss of turgor, impaired mitosis, and a reduced rate of photosynthesis due to drought stress limits overall plant growth (Jaleel *et al.,* 2009). The addition of biochar to soil significantly mitigated such effects of drought on plant height, shoot dry biomass, and stem thickness of wheat. Although, increased growth was seen in control crops also, showing it is not a drought-specific response. However, despite an increase in tiller count when biochar was applied, the difference was not significant. This is in line with results from Olmo *et al.* (2014) who found no significant difference in tiller number between crops grown in a 2% (*w/w*) biochar amended soil and those grown in non-amended soil in a semi-arid Mediterranean climate field study. Rizwan *et al.* (2018) reported a significant increase in tiller number between rice grown in 5% (*w/w*) biochar amended soil and those grown in non-amended soil in greenhouse conditions. Despite similar reductions in wheat and rice biomass at tillering stage (Zhang *et al.,* 2018) under drought stress, rice and wheat have different adaptation mechanisms (Kadam *et al.,* 2015) making a direct comparison difficult. However, considering the use of 60% less biochar in Olmo *et al.* (2014) this study too is a poor comparison. Considering the significant positive effects of biochar seen on other wheat growth attributes, it is possible that the increase seen in this study was not significant due to having too few samples.

Root dry biomass saw no significant change under drought stress compared to the control. Similar results have been reported in maize by Sacks *et al.* (1997). Although, it is generally accepted that the high use of photosynthates by plant roots, over 50% of daily production in some wheat cultivars (Lambers *et al.,* 2002), means a reduction in the rate of photosynthesis leads to reduced root biomass compared to controls in wheat (Liu *et al.*, 2004; Liu *et al.,* 2005; Abbas *et al.,* 2018) with more drought resistant varieties seeing even more of a reduction in root mass due to better carbon partitioning and reduced root respiration (Liu *et al.,* 2004; Liu *et al.,* 2005). Reduced root dry biomass under drought stress has also been reported in other crops (Zeid and Shedeed, 2006; Liu *et al.,* 2011; Rizwan *et al.,* 2018). Considering that a decrease in root biomass, albeit insignificant, is seen, it is possible that the insignificance was due to a low number of repeats. Regardless, the application of biochar saw a significant increase in root biomass compared to those plants not grown with biochar regardless of water treatment.

There was no statistical significance in root-shoot ratio between drought stressed and control crops. This is not supported by the literature, neither by data (Liu *et al.*, 2004; Liu *et al.*, 2005; Chen *et al.*, 2021), or in theory: plant root-shoot ratio varies depending on environmental conditions for the most efficient assimilation and use of resources (Hsaio and Xu, 2000). Under drought stress, plants expend a greater proportion of energy on root growth compared to shoot growth as a deeper more massive root structure can increase water uptake (Ahmad *et al.,* 2018). Low water potential (Ψ) because of drought conditions means it is harder for roots to take up water due to a lower Ψ gradient (Hsaio and Xu, 2000). To combat this and promote water uptake, osmotic adjustment occurs very quickly in the roots re-establishing a Ψ gradient and increasing cell loosening allowing the roots to continue growing while under drought conditions (Hsaio and Xu, 2000). This is not the case in the leaves as osmotic adjustment occurs more slowly, leading to slowed growth. This means under drought conditions, root:shoot ratio should increase (Hsaio and Xu, 2000). It should be noted, however, although insignificant, there was an increase in root to shoot ratio from 0.124 ± 0.005 to 0.135 ± 0.008 in drought stressed wheat compared to the control which is in line with significant data from Chen *et al.* (2021) who saw a similarly proportionate increase in root to shoot ratio from 0.36 ± 0.06 to 0.39 ± 0.1. This suggests that with more repeats, the difference may have been significant. Biochar had no significant effect on the root-shoot ratio of wheat. This is in line with results for barley (Prendergast-Miller *et al.,* 2014) and maize (Zheng *et al.,* 2013). Bista *et al.* (2019) also showed negligeable increases and decreases in root-shoot ratio in wheat depending on biochar application rates compared to the control, however, no significance data is reported.

The mitigation of the effects of drought on plant height, root and shoot biomass, and stem thickness in wheat by application of biochar is supported by numerous studies (Abbas *et al.,* 2018; Haider *et al.,* 2020; Khan *et al.,* 2021; Zaheer *et al.,* 2021). Increased soil water retention and plant water availability because of biochar application, as shown by Ma *et al.* (2016) and Razzaghi *et al.* (2020), allowed for an increased rate and efficiency of photosynthesis leading to increased carbon fixation. However, reduced relative water content in drought stressed wheat plants grown with biochar compared to those not grown with biochar suggests a reduction in plant water availability because of biochar application. Though, this is likely not the case due to the significant increases in growth parameters as well as the significantly higher quantum efficiency of PSII in the plant's grown with biochar indicating that despite the reduced relative water content in the biochar grown wheat, the plants were not more stressed. Instead, it is likely that the overall higher biomass and therefore greater leaf surface area allowed for greater transpiration and so greater water use. It is also possible that the increased water availability due to biochar led to increased water uptake by the wheat causing the more rapid depletion of water seen from the biochar amended soil (Blackwell *et al.,* 2010). Such a loss of water would not have been due to increased soil evaporation due to biochar (Zhang *et al.,* 2016; Zhao *et al.,* 2021).

Because the stressed plants were not watered and held at a set field capacity after drought initiation, towards the end of the experiment the plants grown with biochar may have begun experiencing a water deficit, leading to reduced relative water content compared to the plants grown without biochar. Had the plants continued to be exposed to drought then the biochar may have led to more severe drought stress more quickly compared to the wheat grown without biochar. This could make biochar application in non-irritated areas where extended periods (>4 weeks) of drought occurs counter-intuitive to mitigating drought stress and increasing yield. Although, the differences in water availability between field and pot should be considered here with field having a much greater reserve of water due to the depth of the soil. However, it has positive implications for reducing irrigation water use. By using a system that employs reduced irrigation frequency and deficit irrigation, a practice that involves accepting reduced crop yields for the benefit of increased profits by applying water below the evapotranspiration requirements, reducing water use, English and Nakamura *et al.* (1989) significantly improved the water use efficiency of wheat in a sandy soil. They found that a four-week interval between irrigating at 58% of consumptive use led to the best water use efficiency while still achieving 95% of the crop yield of the fully irrigated crops with 36% less water use. If biochar application was incorporated with such practices, yield losses could be mitigated or reversed completely by improving resistance to drought stress over the four-week period of no irrigation by increasing availability of water to plants along with improvements to plant-soil interactions such as nutrient uptake (Agegnehu *et al.,* 2015).

Conclusion

Overall, biochar is an effective soil amendment for increasing plant growth and health by improving plant-water and plant-soil interactions in sandy soils under any water conditions. In drought conditions, biochar can mitigate drought stress, improving photosynthetic efficiency, further improving wheat growth in a sandy loam. However, it is possible that in areas where long term drought occurs with limited irrigation availability that biochar could be detrimental during extended periods of drought due to the increased water availability and so increased rate of water usage. While biochar would be beneficial for at least four weeks of drought, much longer could lead to rapid deterioration of plant health and increased drought stress due to the more rapid depletion of water. Therefore, further research is needed to determine how long of a period of drought biochar can still be of benefit. In irrigated systems, biochar could be used alongside techniques to improve water use efficiency without seeing reduced crop yields, however, research with such techniques is needed to accurately determine the effects of biochar on such a system. The increased production and use of biochar could be an effective way of sequestering carbon into a stable form, increasing soil carbon and reducing atmospheric carbon while promoting increased wheat yields maintaining food security reducing our dependence on energy intensive agrochemicals through improving nutrient availability and reducing leaching.

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